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Monoclonal Antibody Therapy
September 12th

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Protein Engineering

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About me

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- MSc molecular biotechnology - KTH (2007)
- PhD proteomics/protein engineering - KTH (2012)
- Postdoc antibody engineering - Toronto (2014-16)
- Researcher protein/antibody engineering − KTH (2016-)

- Current research interests
  - Engineering affinity proteins for use in basic research, structural biology, immunology, diagnostics and therapy of cancer, infectious disease etc.

MASTER THESIS PROJECTS AVAILABLE!

Top ten now and before

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2000-2011

Billion USD (11 yrs)			
#1	**Lipitor**	121	Pfizer
#2	**Plavix**	74	BMS/Sanofi
#3	**Advair**	58	GSK
#4	**Zyprexa**	50	Eli Lilly
#5	**Enbrel**	45	Amgen/Pfizer
#6	**Nexium**	44	AstraZ
#7	**Singulair**	38	Merck
#8	**Seroquel**	37	AstraZ
#9	**Lovenox**	33	Sanofi
#10	**Humira**	32	Abbott

?

2017

Billion USD (1 yr)			
#1	**Humira**	18	AbbVie
#2	**Rituxan**	9.2	Roche
#3	**Revlimid**	8.2	Celgene
#4	**Enbrel**	7.9	Amgen/Pfizer
#5	**Herceptin**	7.4	Roche
#6	**Eliquis**	7.4	BMS/Pfizer
#7	**Remicade**	7.2	J&J/Merck
#8	**Avastin**	7.1	Roche
#9	**Xarelto**	6.6	Bayer/J&J
#10	**Eylea**	6.0	Bayer/Regeneron

?

Small molecule
Antibody
Other biological

https://www.genengnews.com/the-lists/

Today

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- Antibody structure and function
- Antibody generation, humanization, *in vitro* selection
- Pros and cons of antibody therapy

Key concepts

---------- BREAK ----------

- Mechanisms of action
- Effector functions
- Bispecific antibodies, checkpoint inhibitors, immunomodulators
- Antibody engineering
- Fc-fusions
- Alternative scaffold binders

Overview of current and future approaches

Vaccines and antibodies

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In 1796 Edward Jenner discovered that cowpox or vaccinia induced protection against human smallpox - an often fatal disease

In 1890 Emil von Behring and Shibasaburo Kitasato discovered that the serum of vaccinated individuals contained substances that specifically bound to the relevant pathogen, which they called antibodies

1901

The magic bullet

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Paul Ehrlich reasoned (1897) that if a compound could be made that selectively targeted a disease-causing organism, then a toxin for that organism could be delivered along with the agent of selectivity. Hence, a "magic bullet" - an ideal therapeutic agent - would be created that killed only the organism targeted

The concept of a "magic bullet" has to some extent been realized by the development of antibody-drug conjugates (a monoclonal antibody linked to a cytotoxic drug) used to direct cytotoxins to targets (e.g. cancer cells)

Monoclonal antibodies (hybridoma), Milstein and Köhler 1975

1984

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Antibody structure and function

Adaptive immunity

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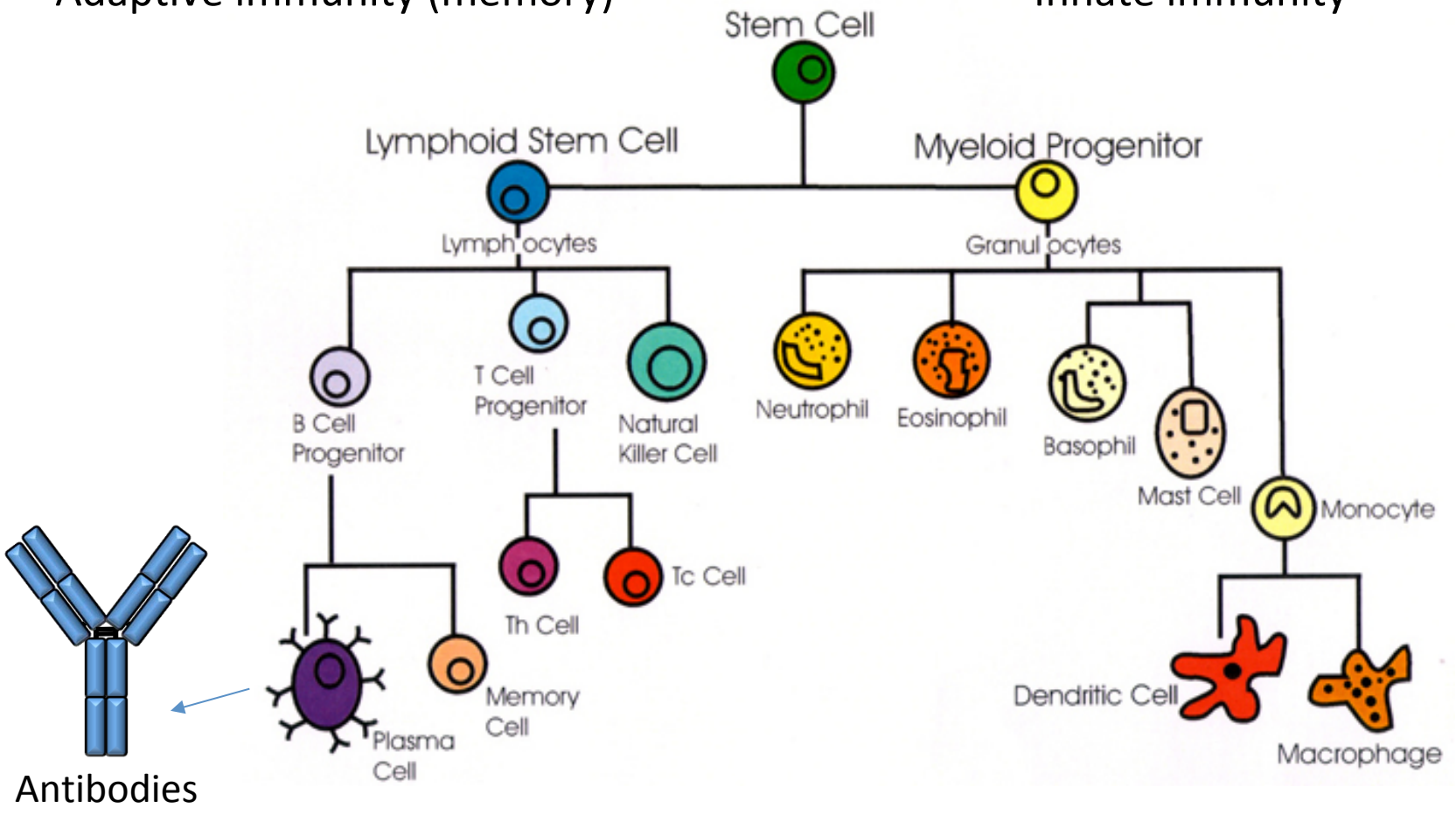
Adaptive immunity (memory)

Innate immunity



Clonal selection

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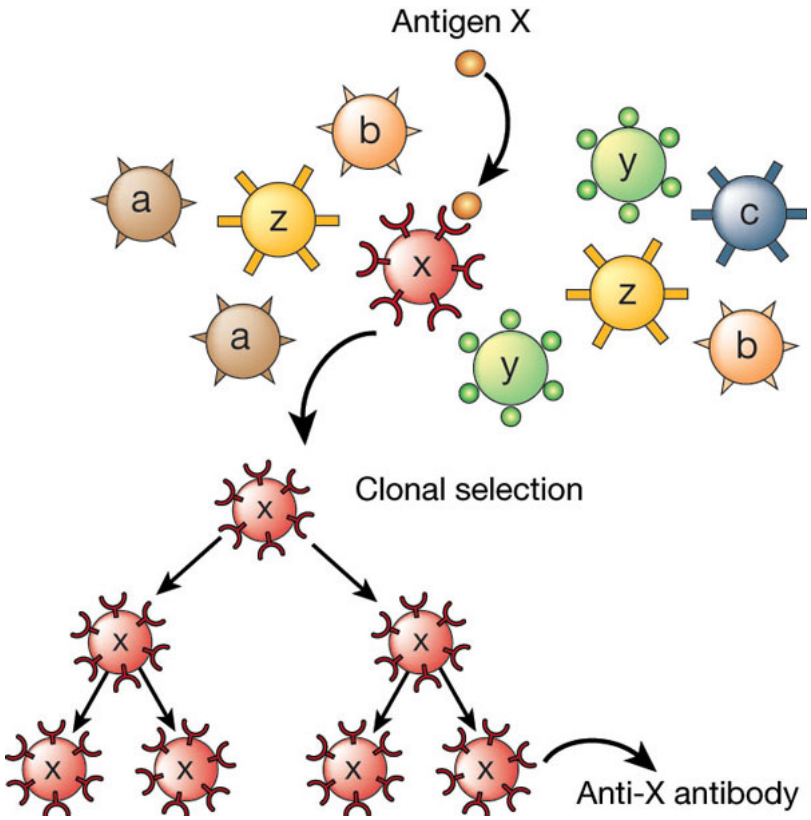
Clonal selection of lymphocytes is the central principle of adaptive immunity



Central tolerance: deletion of lymphocytes specific for self antigens present in generative (primary) organs

Peripheral tolerance: deletion or anergy of lymphocytes that recognize self antigens in peripheral tissue

Monoclonal antibodies (mAbs)

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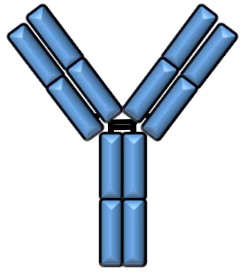
- Monoclonal antibodies are:
  - **Monospecific** and **identical** because they are derived from a single parent cell
  - (*Traditionally*) Produced by fusing a B-cell secreting the desired antibody with a myeloma cell capable of growing indefinitely in culture
  - Targeted to the same epitope with the same affinity (identical antigen-binding sites)



Antibodies – multiple functions

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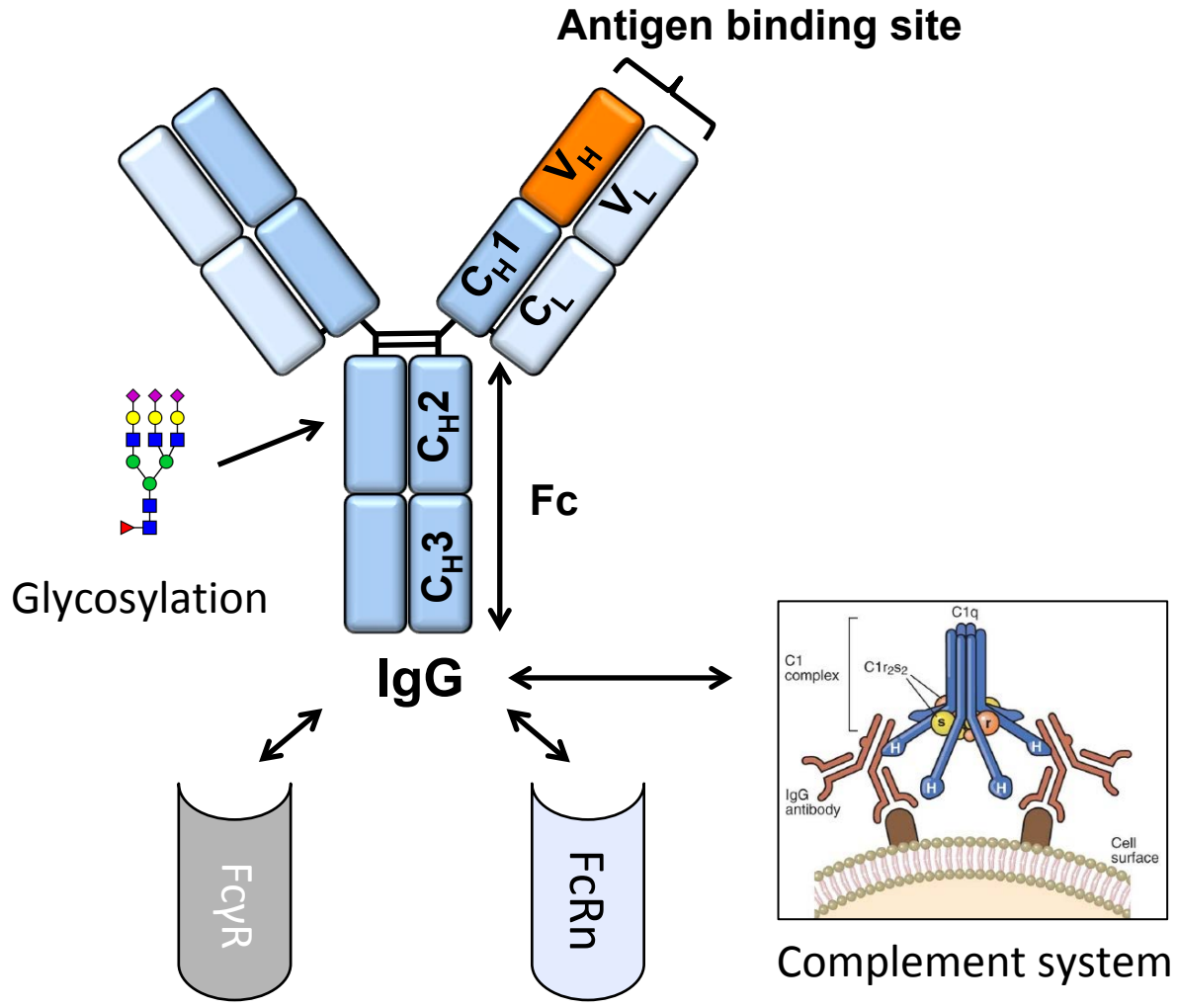
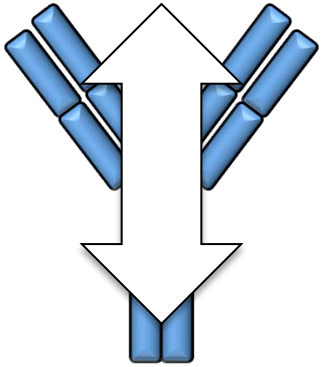


Complement system

Fc-gamma receptors (FcγR)
Neonatal Fc-receptor (FcRn)

Target binding



Effector functions

Antibody domains and CDRs

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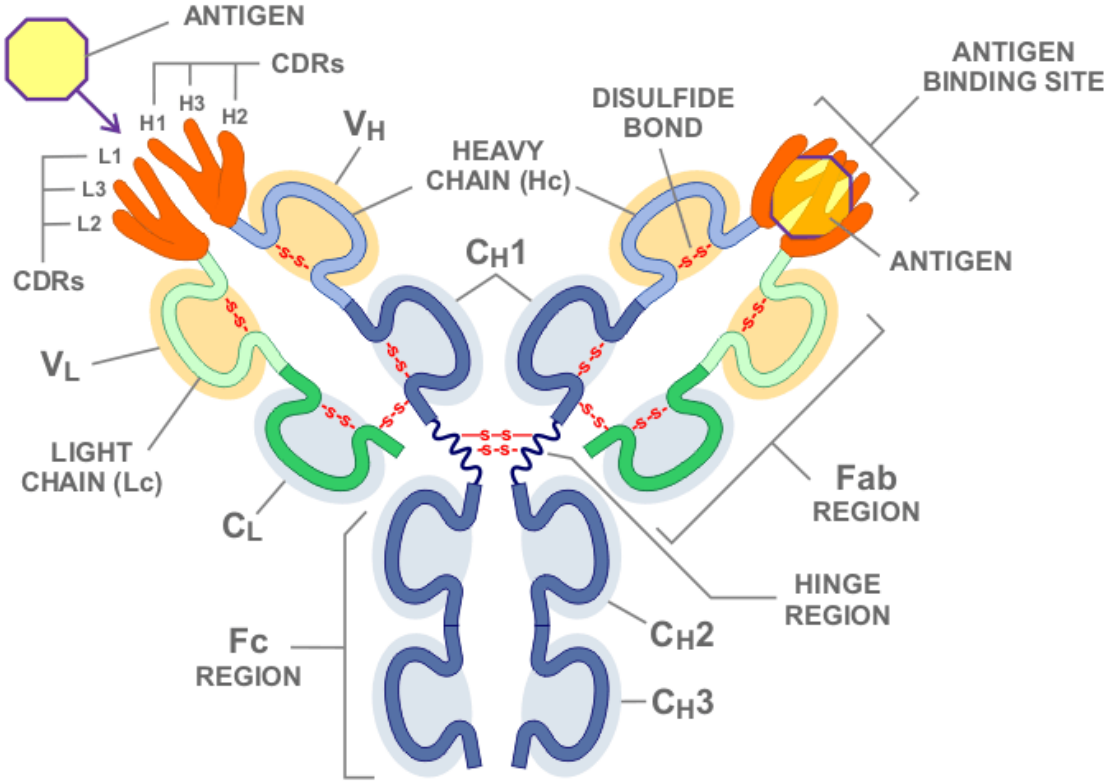

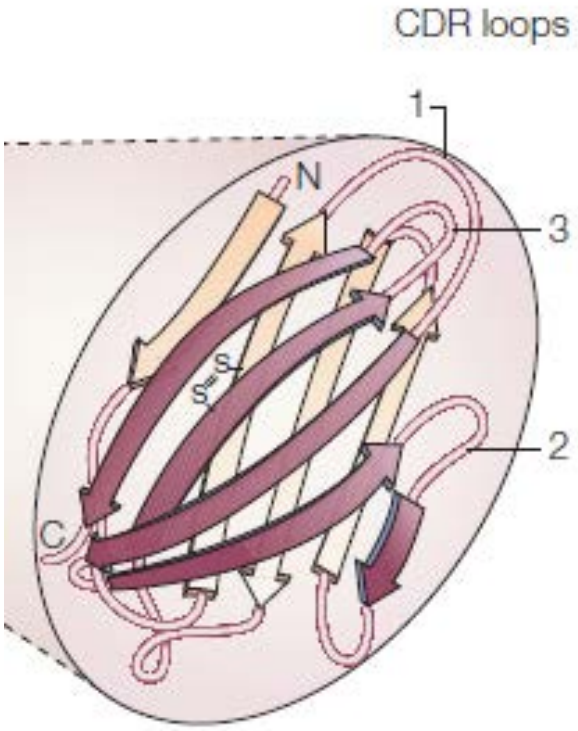

CDR: complementarity determining region

- Normal IgGs are ≈150 kDa tetramers comprising pairs of identical heavy and light chains
- Highly selective antigen binding is mediated by the variable domains in each of the two Fab-arms
- The Fc region mediates effector functions including cytotoxicity (cell-mediated or complement dependent), phagocytosis and serum half-life

Antibody diversity

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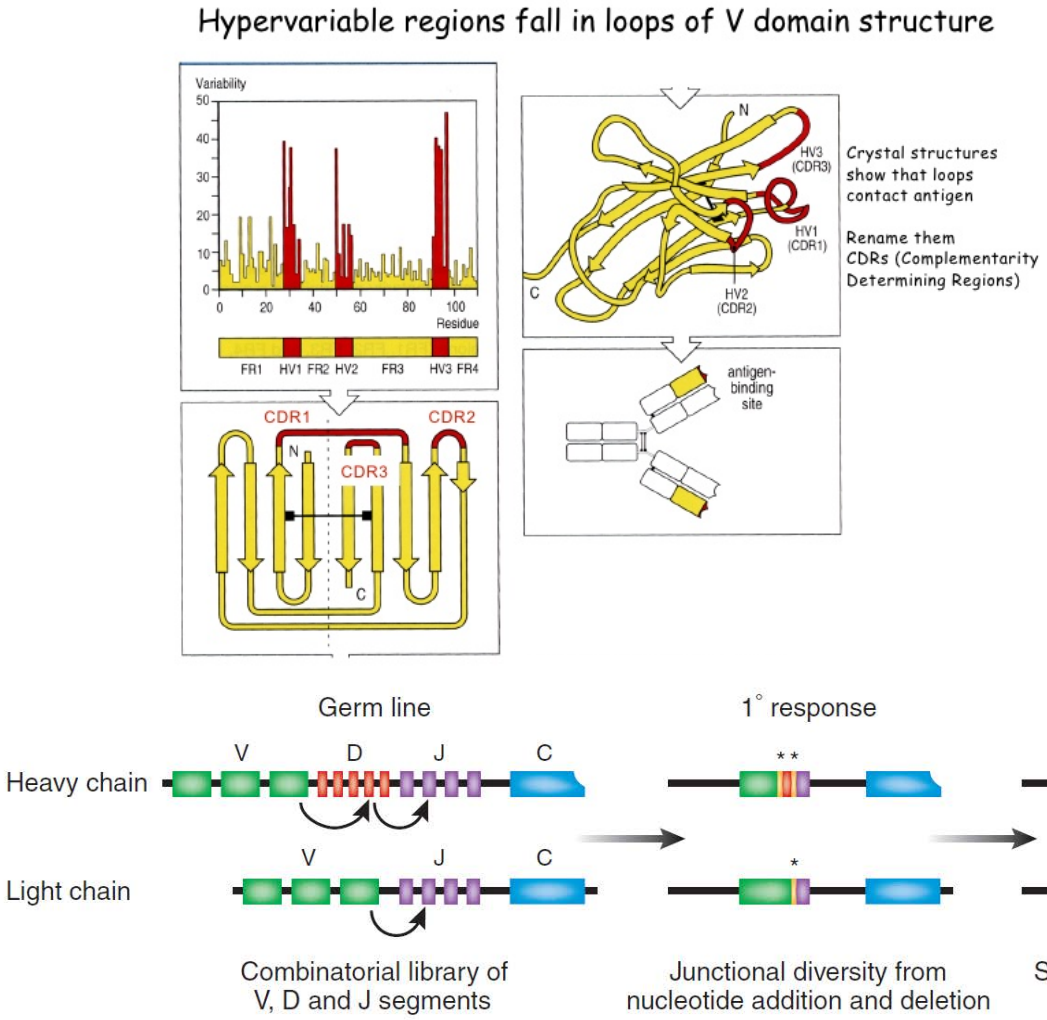

- Each lymphocyte generates a unique antigen receptor by rearranging its receptor genes
- Combinatorial diversity

Hybridoma technology

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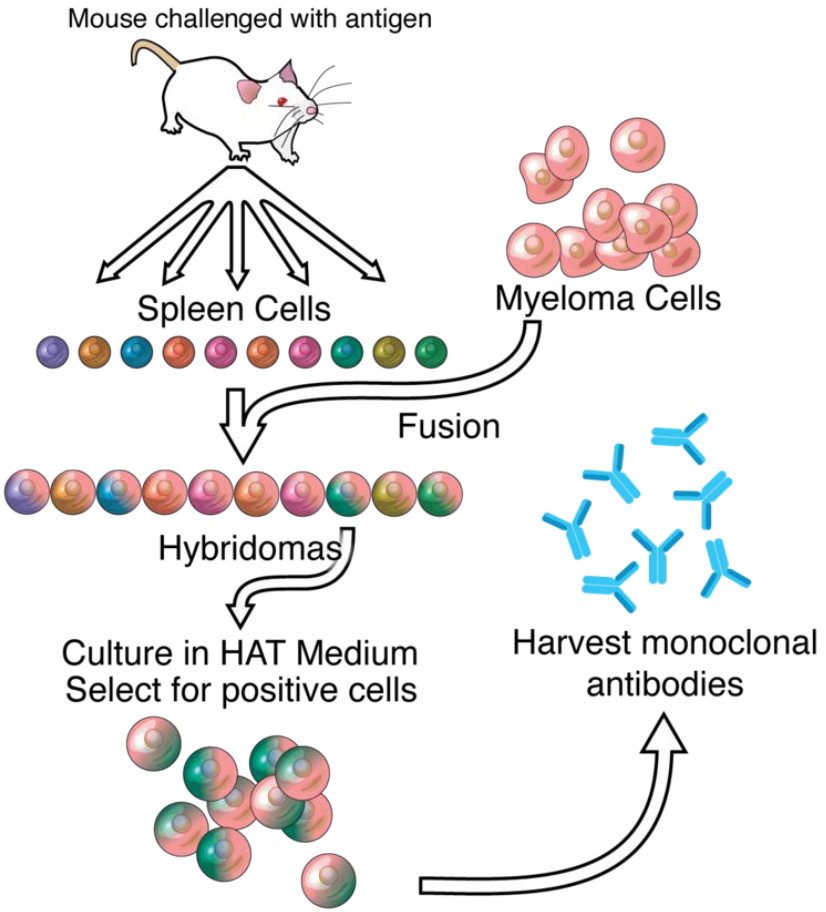
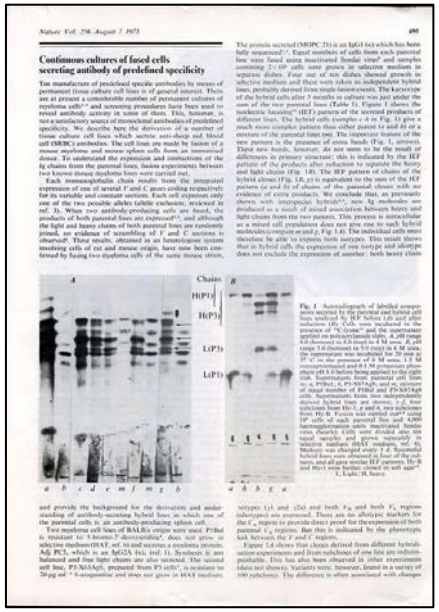
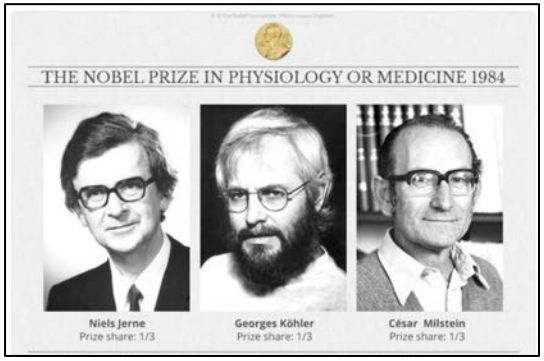
Immunization with antigen + adjuvant, wait several months
Collect B-cells from spleen and fuse with immortal tumor cells



Milstein and Köhler 1975





"for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies"

Antibody humanization

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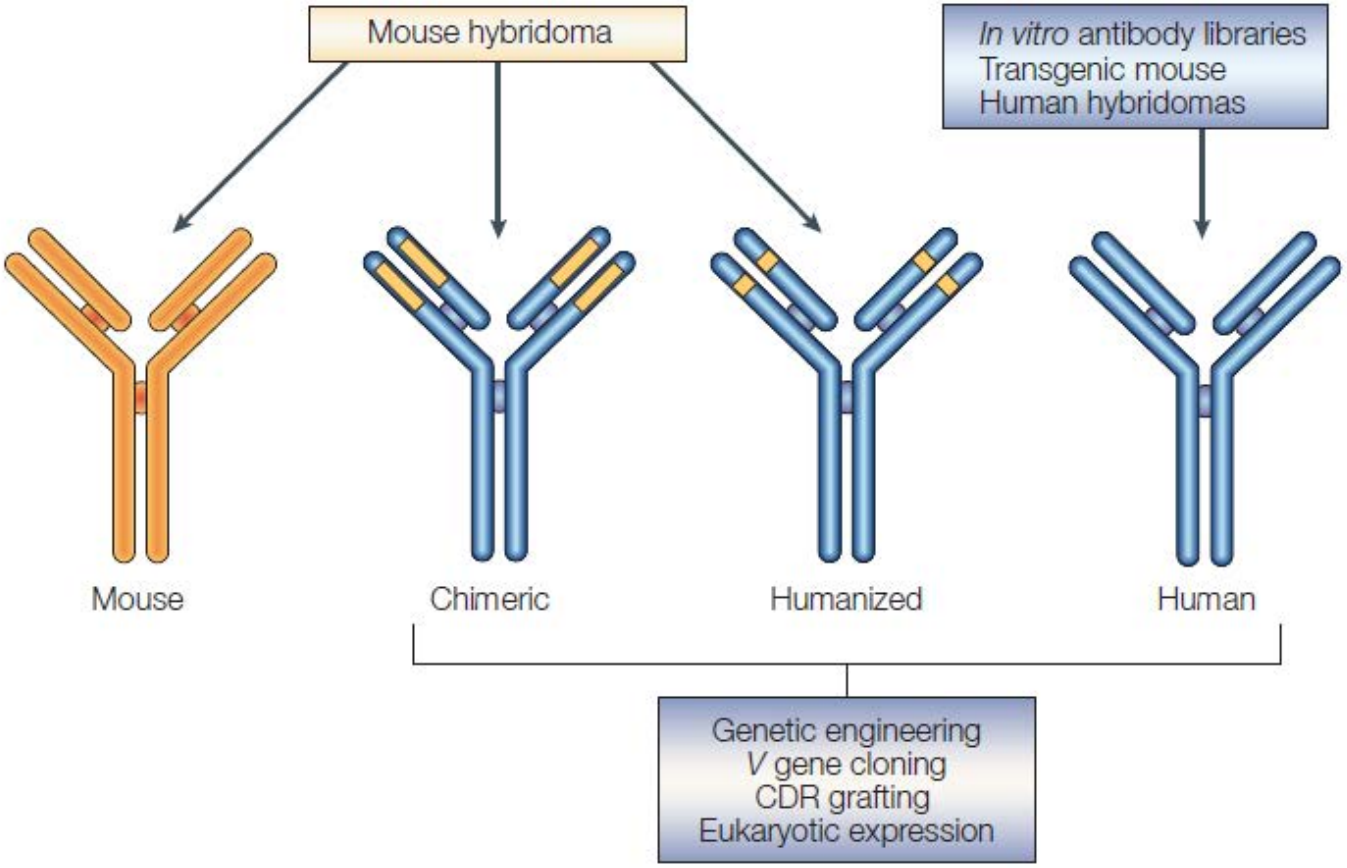

-omab
tositumomab

-ximab
infliximab

-zumab
trastuzumab

-umab
adalimumab

„HAMA": human anti-mouse antibodies
- loss of functional activity of the therapeutic
- induction of side effects
- interference in immunoassays

Evolution of antibody therapeutics

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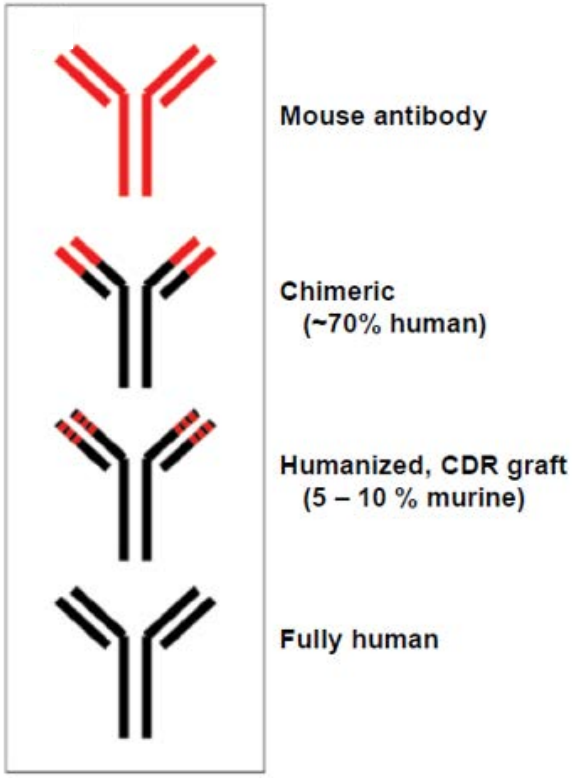
- Mouse monoclonal antibodies
  - Hybridoma technology
  - Major limitations: immunogenicity, lack of effector functions, short serum half-life
- Antibody chimerization and humanization (mid 1980s)
- Human antibodies (1990s)
  - Large phage display libraries (human antibody fragments)
  - Transgenic mice (human immunoglobulin genes)
- Evolving technologies (2000s)
  - yeast, ribosome, mRNA, mammalian and *E. coli* display libraries
  - Direct cloning of human antibodies from human blood- or bone marrow-derived cells



30 years of mAb/Fc-fusion approvals

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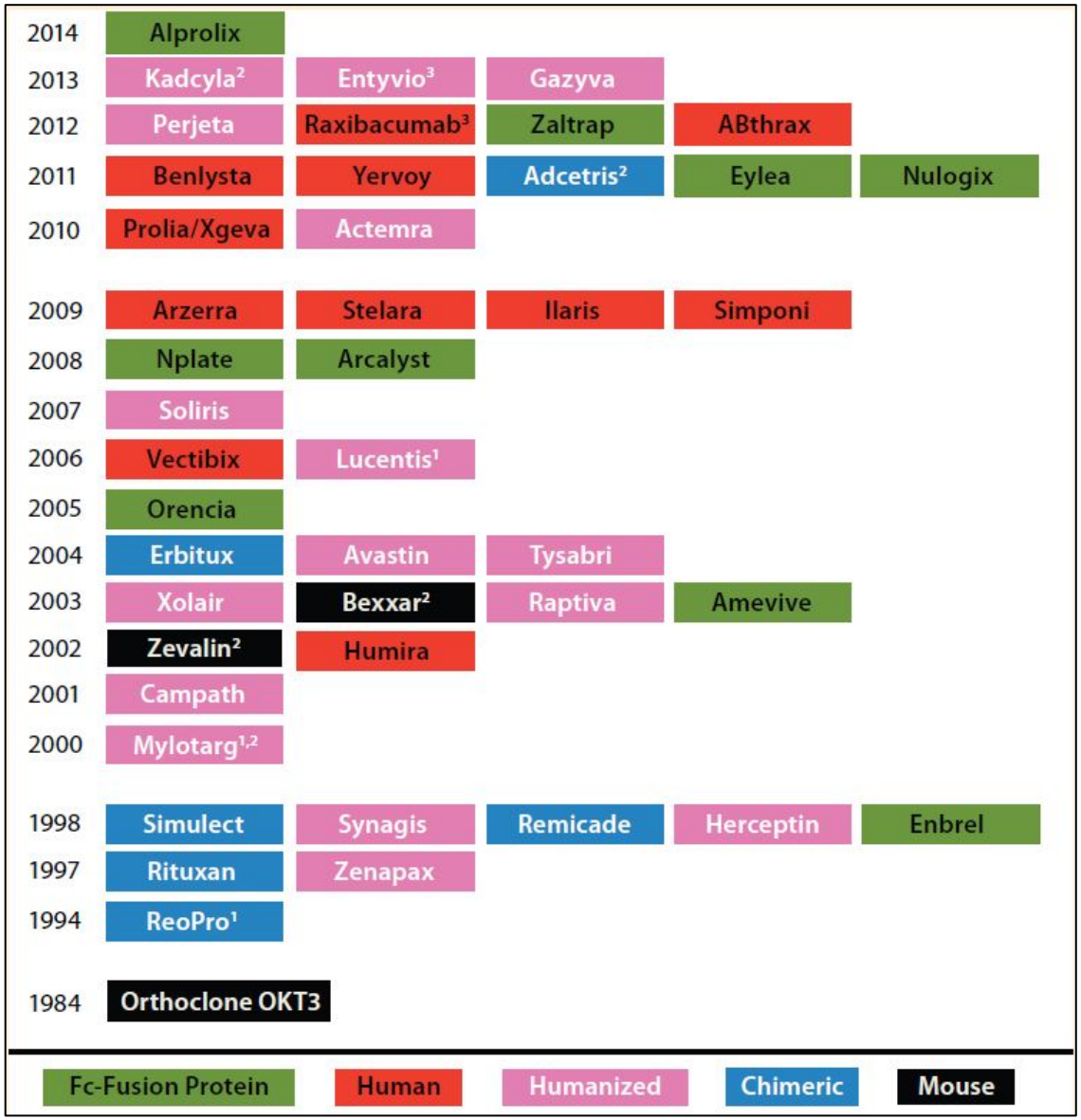


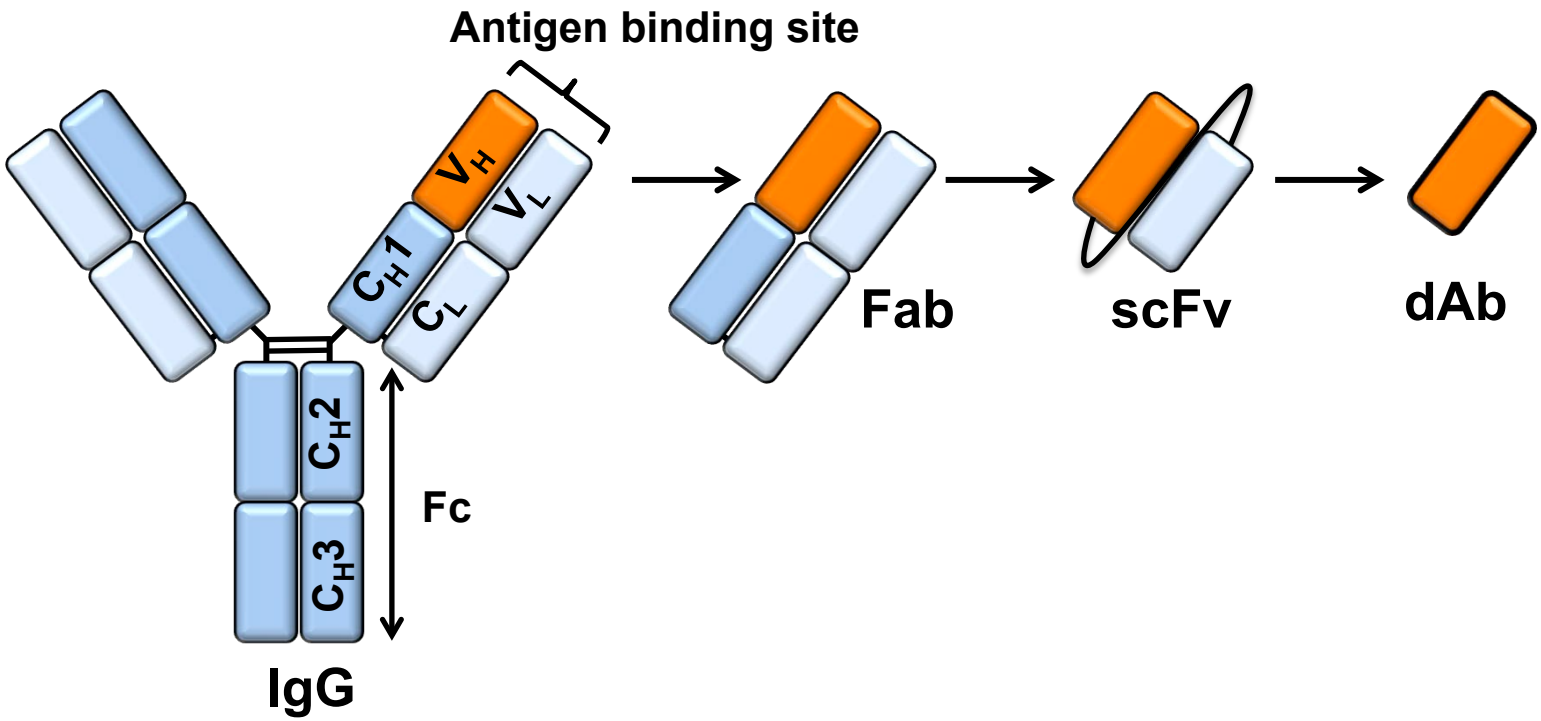
Oncology
Inflammation

>75 FDA/EMA-approved products
ca. 300 in development

Rapidly expanding market

Market expected to reach
125 billion USD in sales by 2020
(almost +50 % in 3 years!)

Antibody fragments



Fab = fragment antigen binding
scFv = single-chain fragment variable
dAb = domain antibody

Fc = fragment crystallizable

Fully human mAbs

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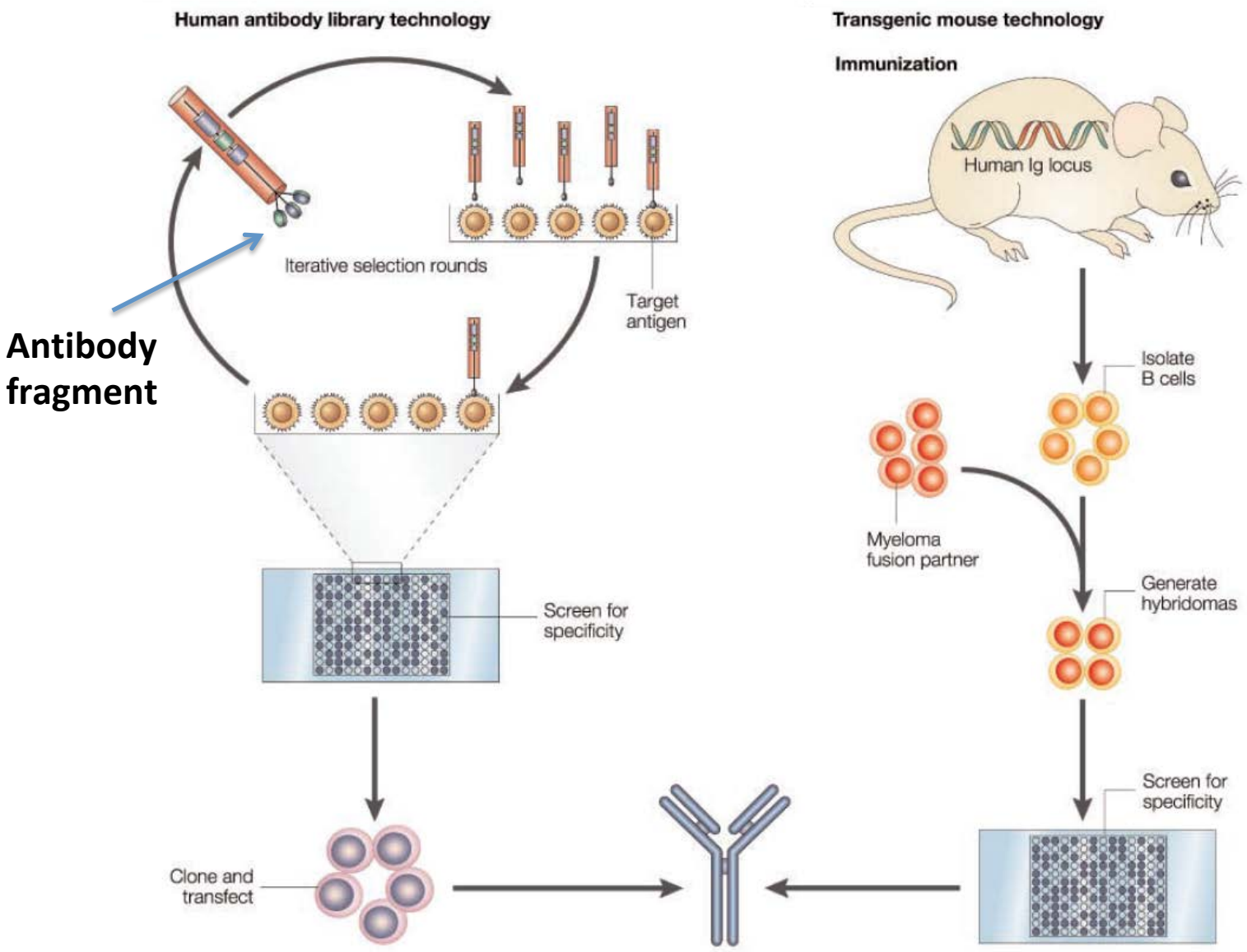

Synthetic antibody libraries

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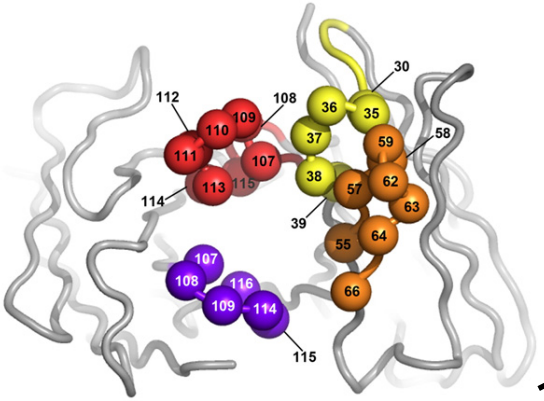
Library design



CDR-L3

105	106	107	108	109	114	115	116	117
Q	Q	X	X	X	X	PL	FI	T

CDR-H1

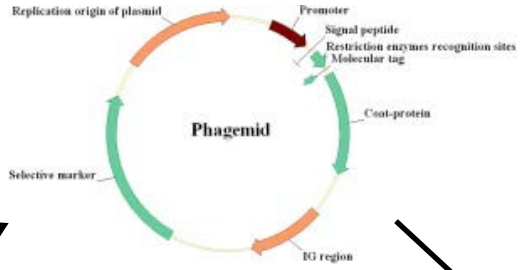
30	35	36	37	38	39
IL	YS	YS	YS	YS	IM

CDR-H2

55	56	57	58	59	62	63	64	65	66
YS	I	YS	PS	YS	YS	GS	YS	T	YS

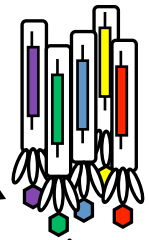
CDR-H3

105	106	107	108	109	110	111	112	113	114	115	116
A	R	X	X	X	X	X	X	X	AG	FILM	D



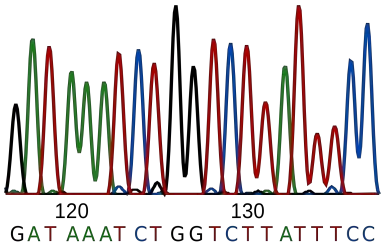
***In vitro* selection**

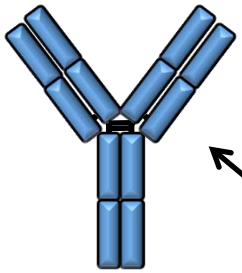
Antibody variants



Antigen

DNA-sequencing





***In vitro* evolution**

Cloning of mAbs from Ebola survivors

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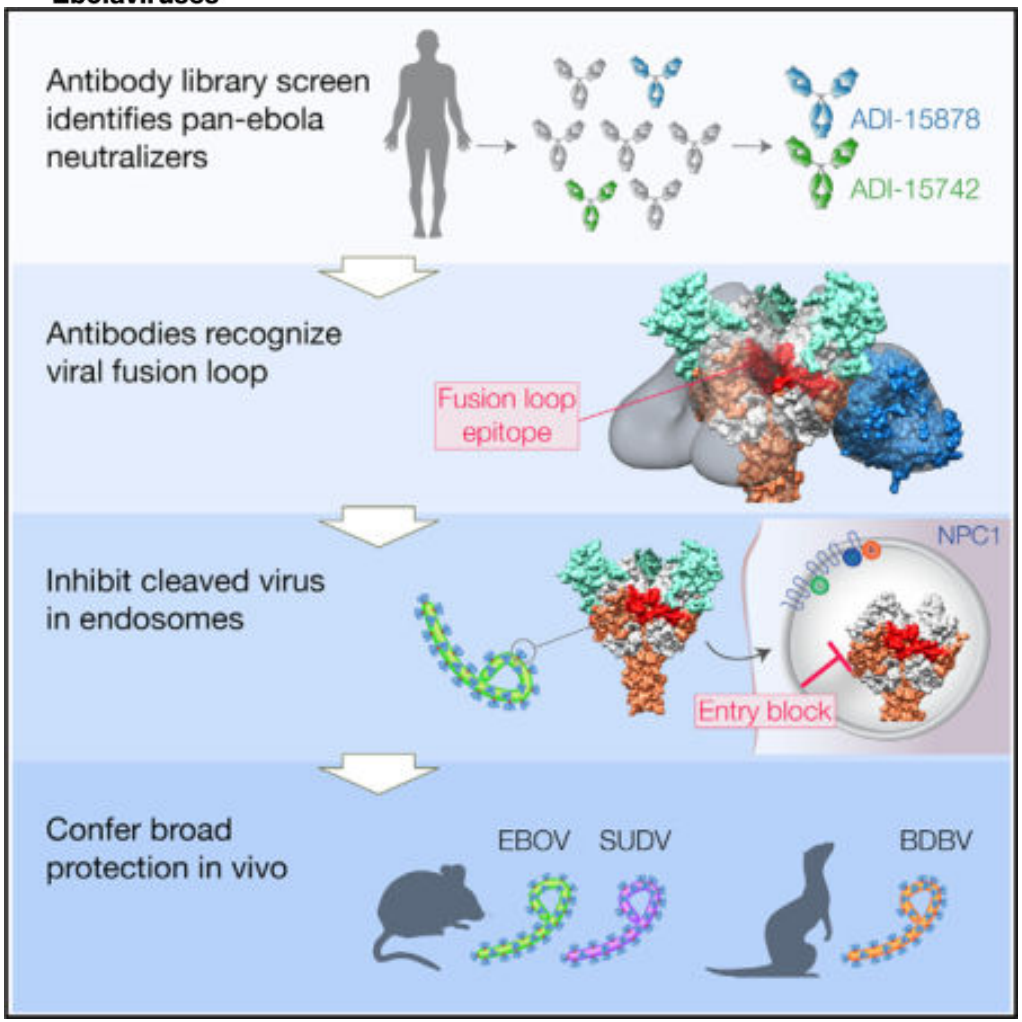
Article

Cell

Antibodies from a Human Survivor Define Sites of Vulnerability for Broad Protection against Ebolaviruses



Pros and cons of mAbs

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Pros and cons of mAbs

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- Good solubility and stability
- Long serum half-life
- High specificity and potency – “any target”
- Low toxicity, typically well-tolerated
- Multiple mechanisms of action
- High success rates
- Well-established class of molecules

- Very poor oral bioavailability (typically i.v.)
- Incomplete absorption following i.m. or s.c. administration
- Nonlinear distribution and elimination
- Immunogenicity (anti-drug antibodies)
- Expensive (mammalian cell production and high doses)
- Limited to extracellular target space/cell surface antigens
- Limited tissue penetration due to large size

Current development

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- Tailor effector functions
- Tailor half-life
- Expand target space (CNS, intracellular targets)
  - Challenging!
- Multiple targets – bi- and multispecifics
- Payloads (e.g. antibody-drug conjugates)
- …
- Omit the antibody entity – alternative protein scaffolds

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BREAK

Mechanisms of action

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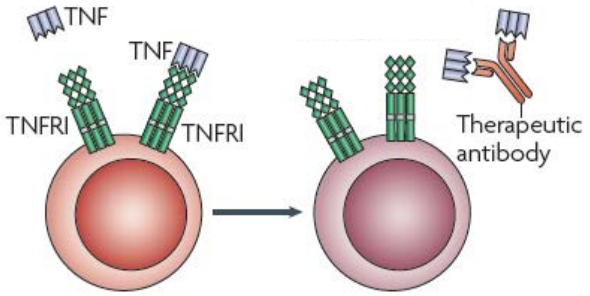
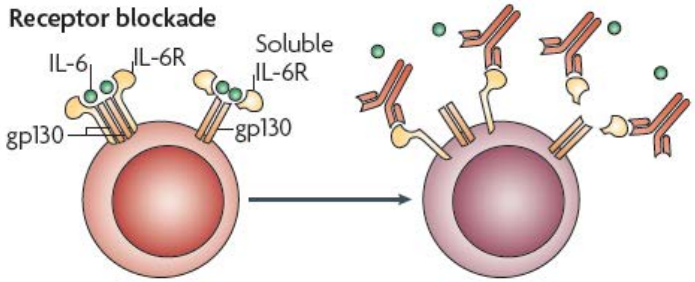
Ligand blockade



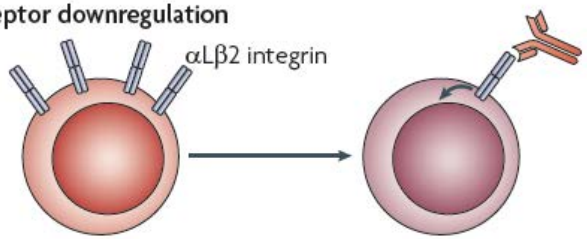
Infliximab*	Belimumab
Adalimumab*	Eculizumab
Golimumab	Mepolizumab
Certolizumab pegol	Reslizumab
Canakinumab	Etanercept‡
Briakinumab	Atacicept‡
Ustekinumab	Alefacept‡
Omalizumab*	

Receptor blockade



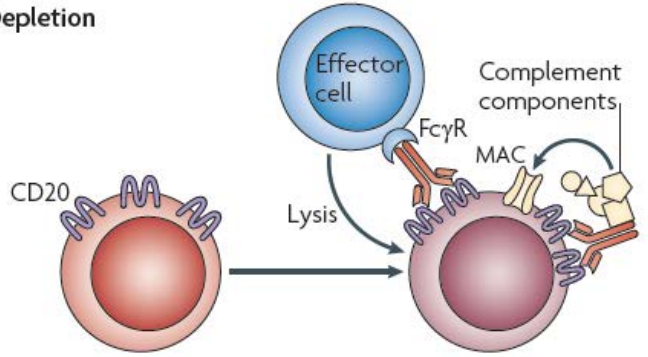
Tocilizumab
Efalizumab*
Natalizumab
Vedolizumab
Abatacept‡

Receptor downregulation



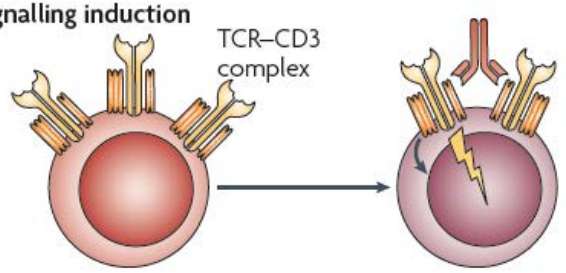
Efalizumab*
Omalizumab*
Otelixizumab*
Teplizumab*
Epratuzumab*

Depletion



Rituximab*
Ofatumumab
Ocrelizumab
GA101*
Alemtuzumab
Muromonab*
Epratuzumab*

Signalling induction



Otelixizumab*
Teplizumab*
Muromonab*
GA101*
Infliximab*
Adalimumab*
Rituximab*

* Several modes of action

Effector functions

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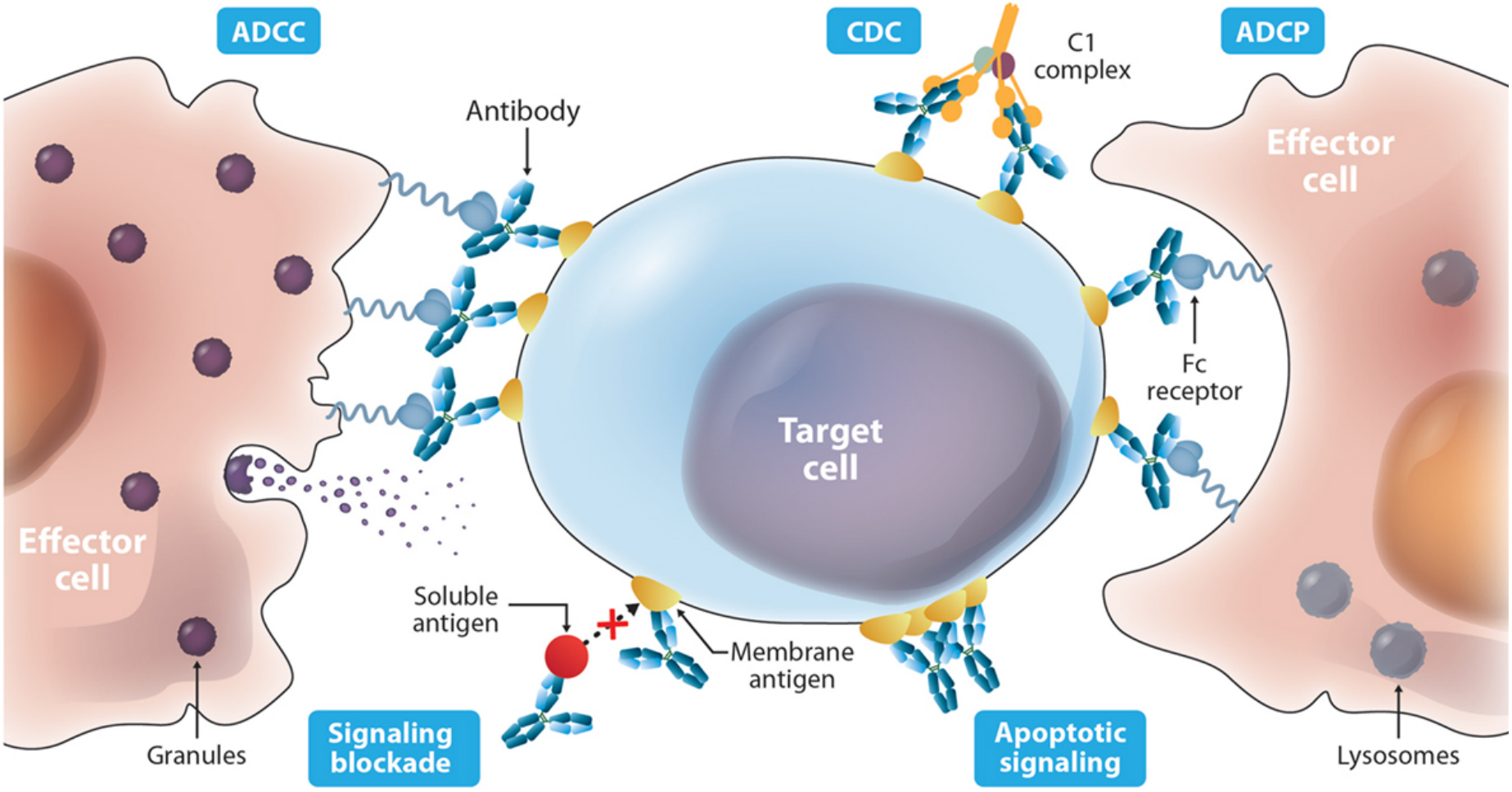

ADCC = antibody-dependent cell-mediated toxicity
CDC = complement-dependent cytotoxicity
ADCP = antibody-dependent cellular phagcytosis

Neonatal Fc-receptor (FcRn)

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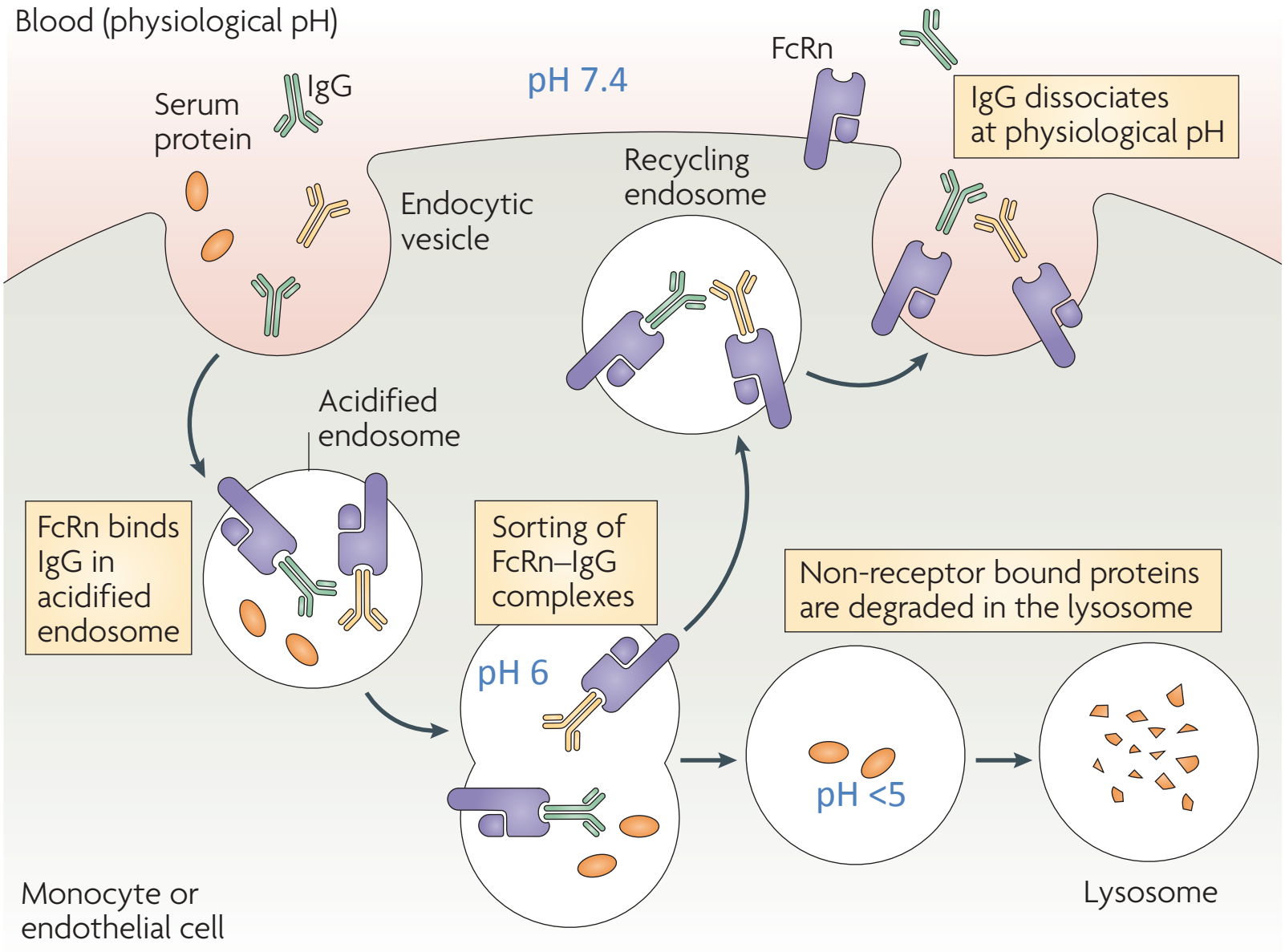

FcRn – optimization of recycling

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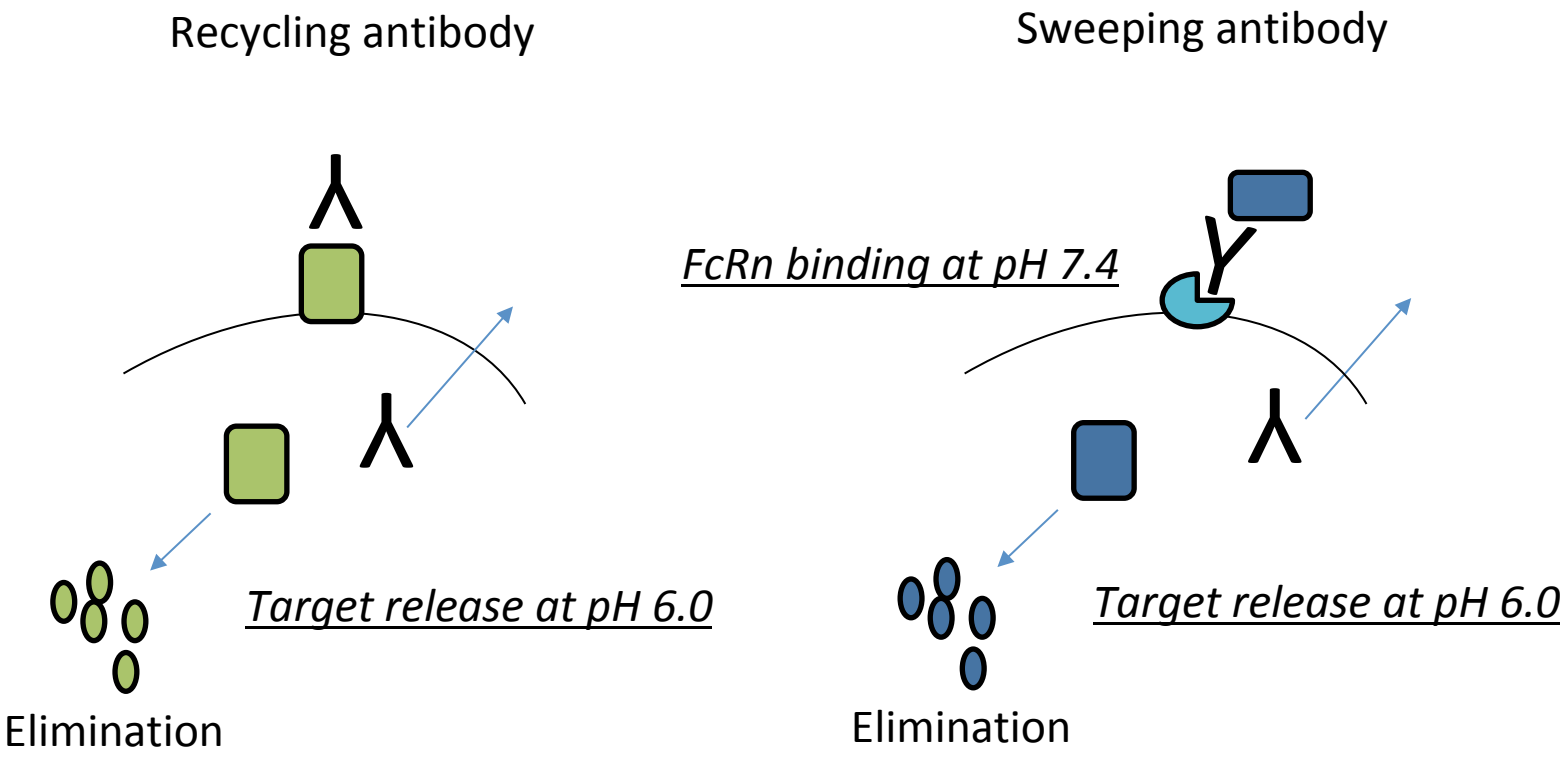

- Engineer target release at pH 6
- Increase the number of cycles in which a mAb binds to and releases antigen for lysosomal degradation
- Many mAbs only bind one antigen molecule during their lifetime in plasma

Immune checkpoint inhibitors

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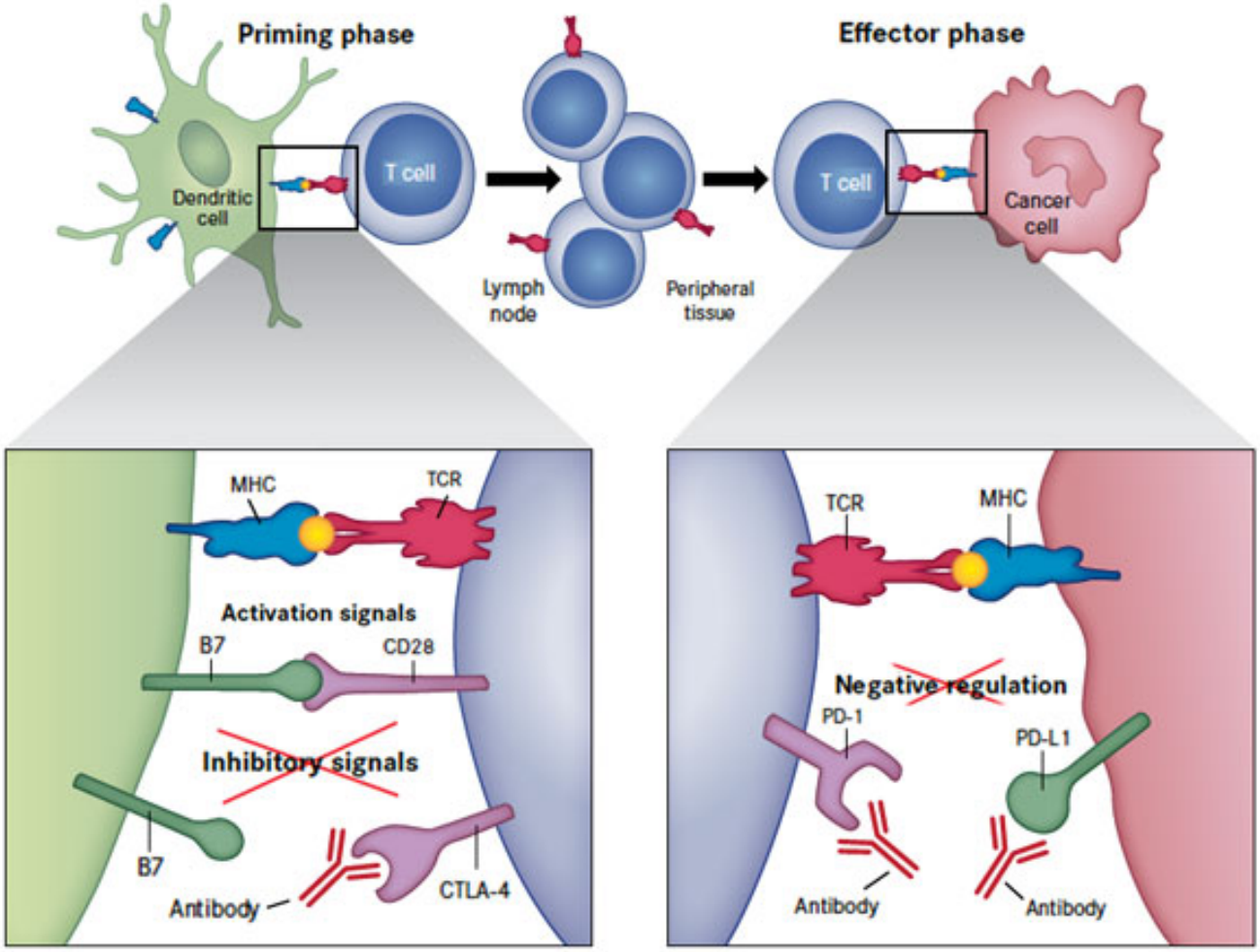

Immune checkpoint inhibitors restore and augment the antitumor immune activities of cytotoxic T-cells by blocking immune checkpoint molecules on T-cells or their ligands on antigen-presenting and tumor cells

Bispecific antibodies

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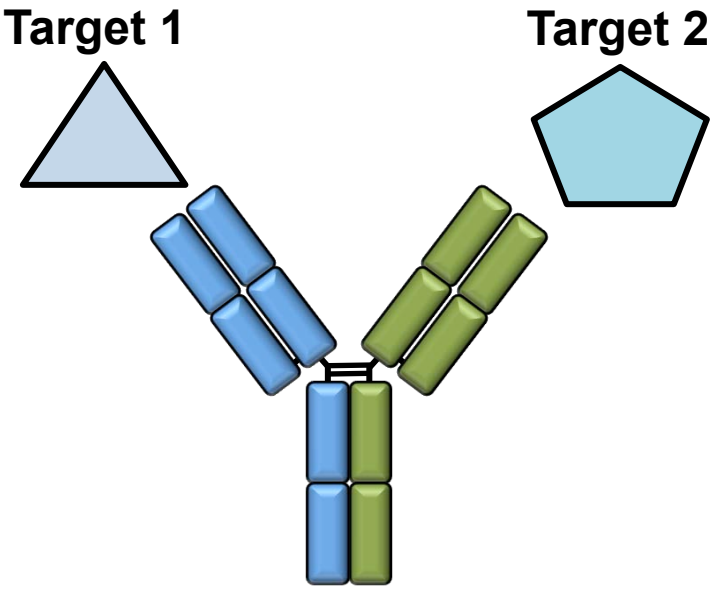
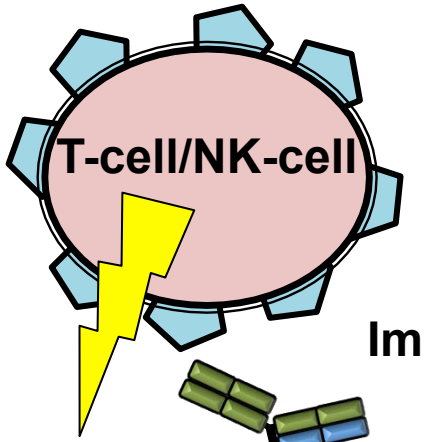
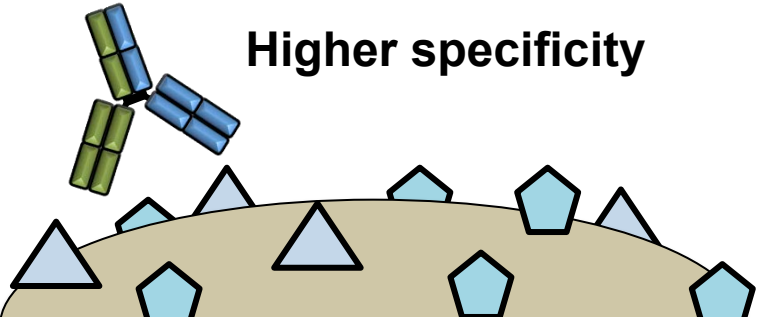


Bispecific IgG

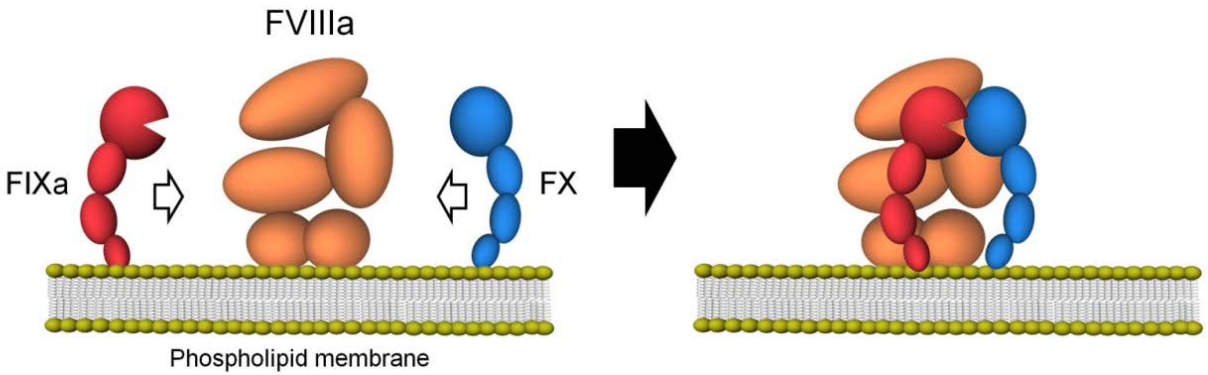


Immune cell recruitment

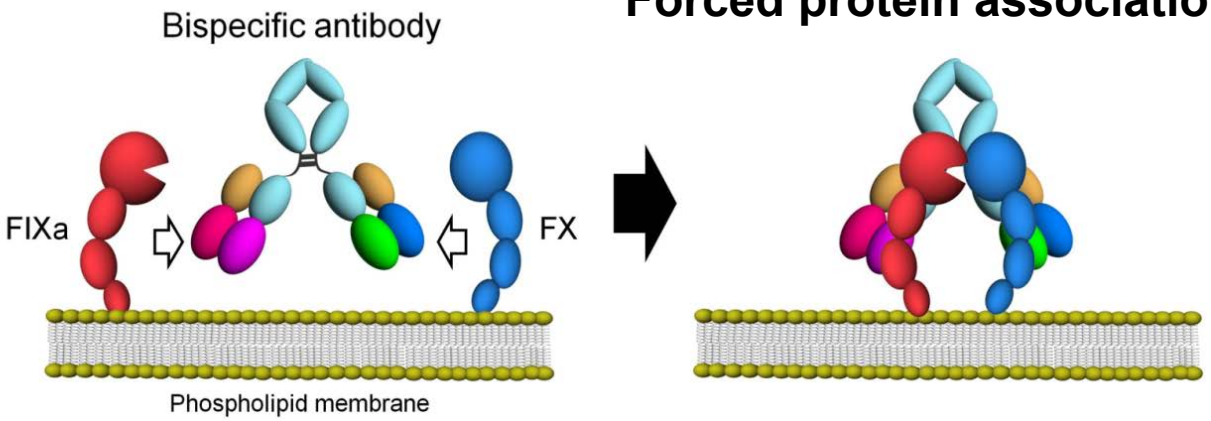
Higher specificity



Bispecific antibodies



Forced protein association



Binds to activated factor IX and to factor X mediating activation of the latter, which is normally the function of VIII

Antibody engineering

KTH VETENSKAP OCH KONST

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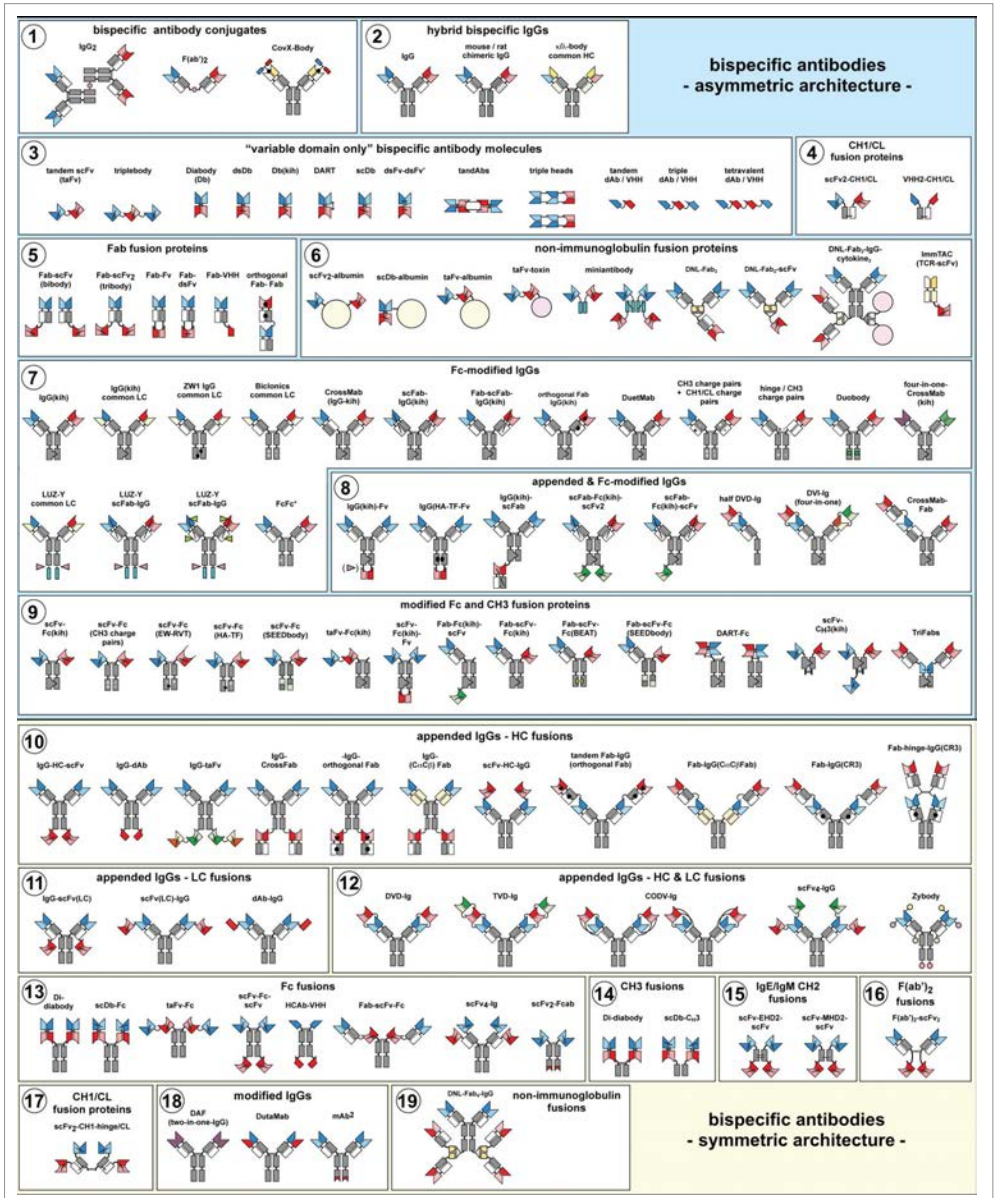

>100 bispecific formats reported

Why so many?

Immunoconjugates

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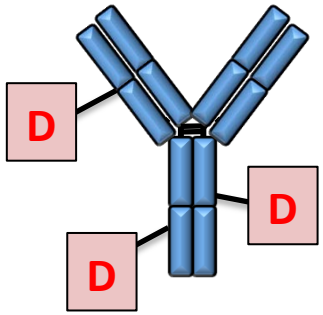
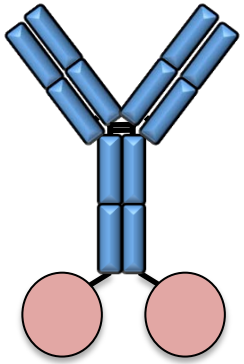
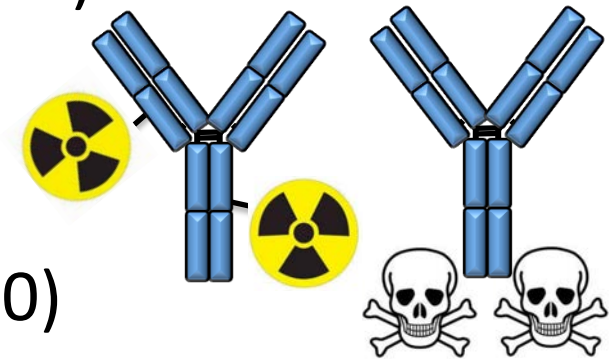
- Antibody-drug conjugates (ADCs)
  - Mylotarg (FDA-approved 2001; discontinued 2010, reapproved 2017) – CD33
  - Adcentris (2011) – CD30
  - Kadcyla (2013) – HER2
  - Besponsa (2017) – CD22
- Immunocytokines (e.g. antibody-cytokine fusion)
  - None yet approved
- Immunotoxins (e.g antibody-toxin fusion)
  - Ontak (discontinued in 2014)
- Radioimmunoconjugates
  - Several approved, e.g. Zevalin (CD20)







Fc-fusions

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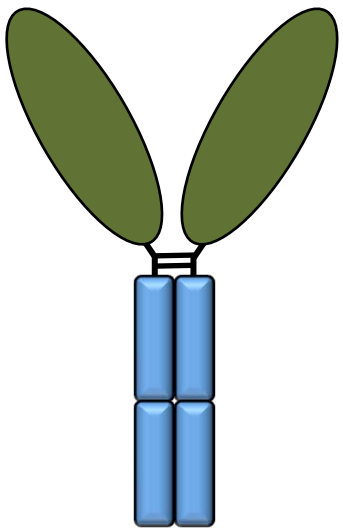

Protein of interest

- Receptor or extracellular domain
- Cytokine
- Enzyme
- etc.

Fc

- FcγR-binding → ADCC
- C1q-bindning → CDC
- FcRn → Half-life

Nanobodies

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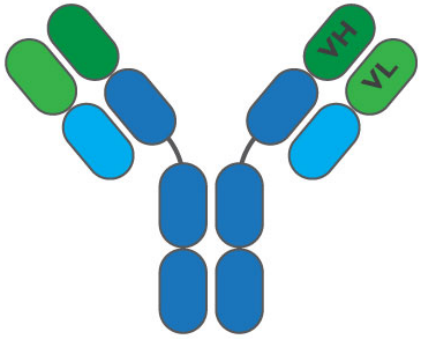
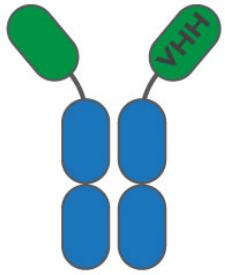


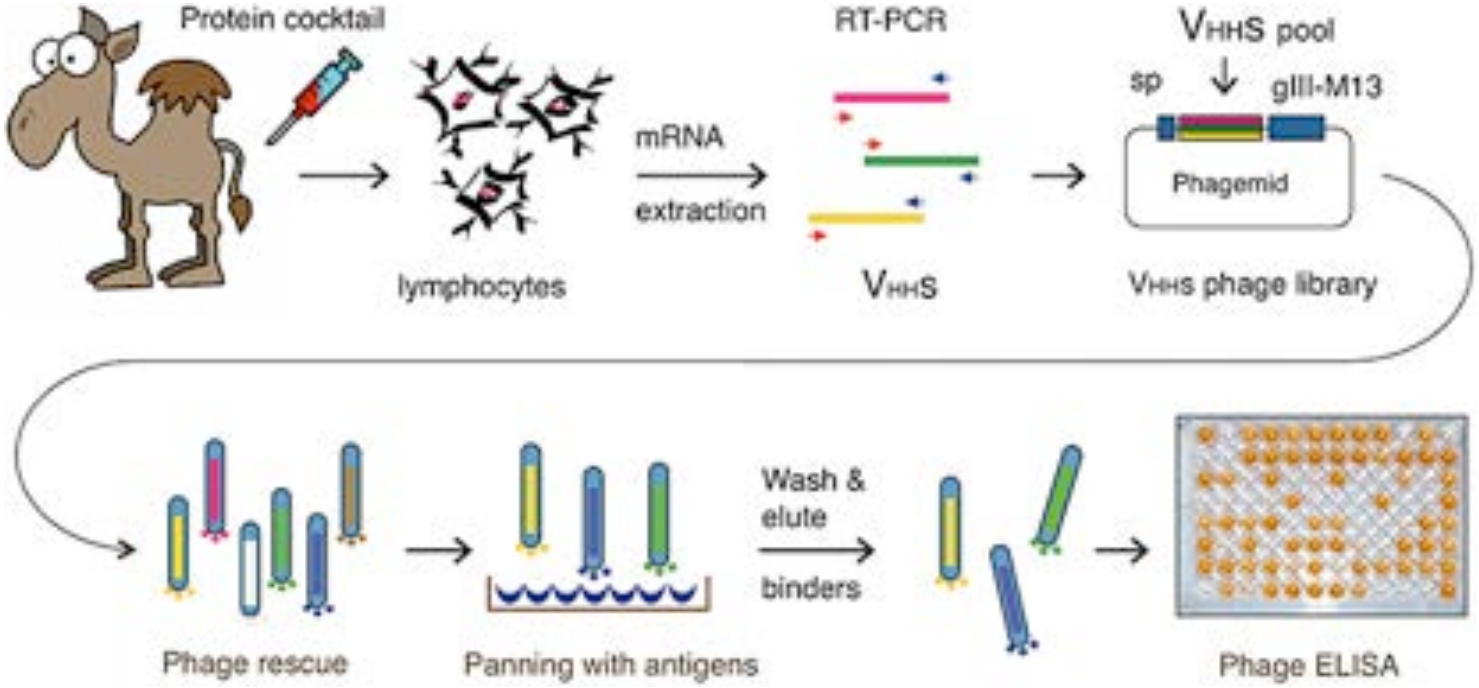
Conventional IgG



Heavy chain-only antibody

VHH/Nanobody (15 kDa)

Ablynx
A SANOFI COMPANY



Non-antibody scaffolds

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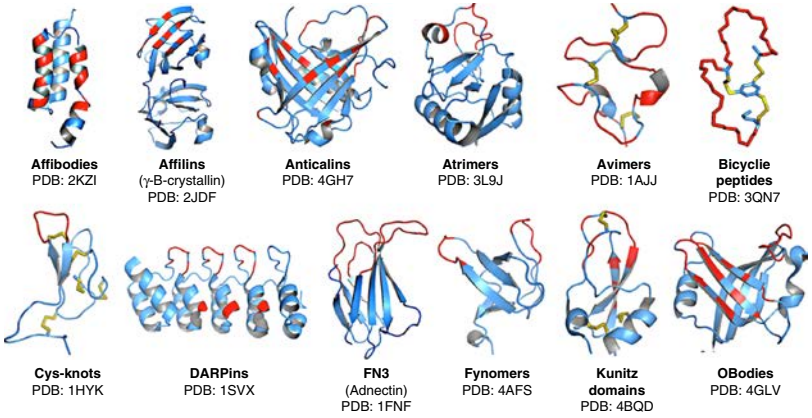
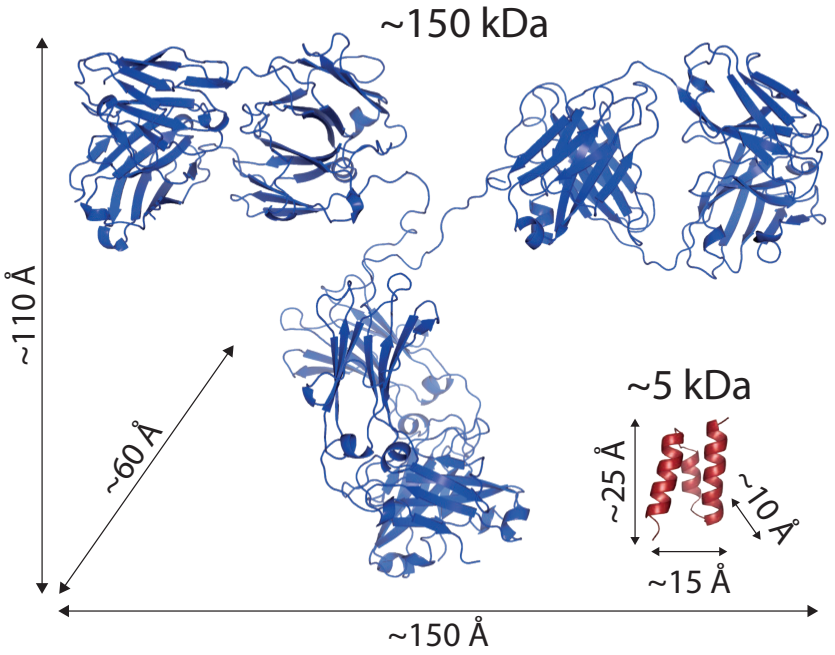


Scaffold	Parental protein	Structure	Randomization	MW (kDa)
Affibodies	Z domain (protein A)	α-helical	Helix randomization	6
Affilins	γ-B-crystallin	β-sheet	Beta-strand randomization	20
	Ubiquitin	α/β	Beta-strand randomization	10
Anticalins	Lipocalin	β-sheet + α-helical terminus	Loop randomization Beta-strand randomization	20
Atrimers	C-type lectin (tetranectin)	α/β	Loop randomization	3 × 20
Avimers	A-domain	Ca^{2+} binding Disulfide constrained	Loop randomization	4
Bicyclic peptides	Peptide	Chemically constrained	Loop randomization	2
Cys-knots	Peptide	β-sheet Disulfide constrained	Loop randomization	4
DARPins	Ankyrin repeats	α-helical + β-turn	Helix randomization Beta-turn randomization	14-21
FN3 scaffolds (Adnectins, Centyrins, Pronectins, Tn3)	Fibronectin (type III)	β-sheet	Loop randomization Beta-strand randomization	10
Fynomers	SH3 domain (fyn kinase)	β-sheet	Loop randomization	7
Kunitz domains	Serine protease inhibitor	α/β Disulfide constrained	Loop randomization	7
OBodies	OB-fold	β-sheet	Loop randomization Beta-strand randomization	12

Drug Discovery Today

KTH:

- **Affibody technology**
- **ADAPT-technology**



Several potential advantages

- Convenient production in bacterial hosts or by chemical synthesis
- Lack of disulfide-bonds
- Chemical and thermal stability
- Easy (re-) folding
- No effector functions
  - May add payloads
- Improved tissue penetration and faster clearance

Non-antibody scaffolds

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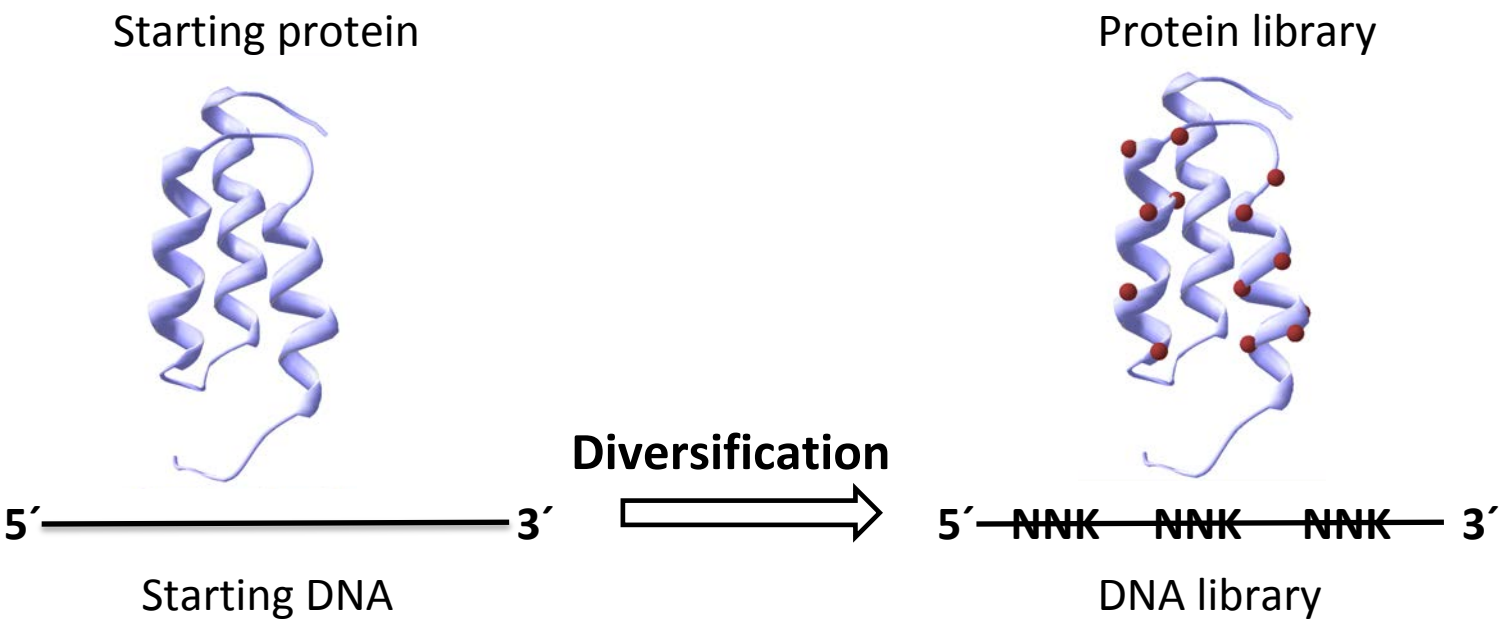
- Combinatorial protein engineering



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